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Micronutrient Group Symposium on 'Micronutrient supplementation: is there a case?'

Is there a potential therapeutic value of copper and zinc for osteoporosis?

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Osteoporosis is almost universal in very old age, and is a major cause of morbidity and mortality in the elderly of both sexes. Bone is lost at a rate of 0·2–0·5 %/year in both men and women after the age of 40–45 years. The causes of age-related changes in bone mass are multifactorial and include genetic predisposition, nutritional factors, endocrine changes, habitual exercise levels and body weight. Bone loss is accelerated to 2–5 % year immediately before and for up to 10 years post-menopause (Heaney, 1986). In women hormone-replacement therapy is effective in reducing the rate of bone loss caused by this peri-menopausal decrease in hormone levels (Smith & Studd, 1993); however, in men and older women (>10 years post-menopause) nutrition plays a key role in the rate of bone loss. One factor contributing to bone loss in the elderly may be a subclinical Zn and/or Cu deficiency, due to a reduced dietary intake of micronutrients and reduced absorption (Thomson & Keelan, 1986). Zn and Cu are essential cofactors for enzymes involved in the synthesis of various bone matrix constituents. Paradoxically, Ca supplementation may accentuate the problem of reduced Zn and Cu levels by impairing the absorption of simultaneously-ingested Zn and the retention of Cu (Snedeker *et al.* 1982; Grekas *et al.* 1988). The present paper will review the current literature on the potential benefits of Cu and Zn supplementation in reducing bone loss, and present new information on the effect of Ca supplementation on Zn and Cu status in post-menopausal women with osteoporosis.

Copper: Zinc: Osteoporosis

The role of copper and zinc in bone metabolism

Zn and Cu are essential cofactors for enzymes involved in synthesis of various bone matrix constituents (Heaney, 1986), and play a particularly important role in the regulation of bone deposition and resorption. Some investigators have reported reduced Zn and Cu concentrations in the bone of patients with senile osteoporosis (Atik, 1983; Conlan *et al.* 1990). However, this finding is not universal, and depends on a number of factors, including site of sampling and the relative proportions of the organic and inorganic bone components (Helliwell *et al.* 1996).

Zinc

Zn is essential for growth. It is well established that Zn is an essential cofactor for enzymes involved in DNA and RNA synthesis (DNA and RNA polymerase), and enzymes involved in protein synthesis. Bone growth retardation is a common finding in Zn deficiency, both experimentally-induced deficiency in growing animals (Guigliano & Millward, 1984) and in children as a result of dietary insufficiency (Walravens *et al.* 1989). Growth retardation is also a characteristic feature of acrodermatitis enteropathica, an autosomal recessive condition that results in Zn deficiency in early infancy (Moynahan, 1974). Studies in

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rats have shown that Zn deficiency results in a reduction in femur Zn concentration (Lowe *et al.* 1991), a reduction in cancellous bone mass and a deterioration of trabecular bone architecture (Eberle *et al.* 1999).

The role of Zn in bone is therefore twofold: (1) Zn plays a structural role in the bone matrix. Bone mineral is composed of hydroxyapatite crystals which contain Zn complexed with fluoride; (2) Zn is involved in the stimulation of bone formation by osteoblasts and inhibition of bone resorption by osteoclasts. Zn is required for osteoblastic activity, directly activating aminoacyl-tRNA synthetase in osteoblastic cells and stimulating cellular protein synthesis. Zn also promotes bone mineralisation through its role as a cofactor for alkaline phosphatase (Yamaguchi, 1998). Studies using mouse marrow cells *in vitro* have demonstrated that Zn plays a role in the inhibition of bone resorption by inhibiting osteoclast-like cell formation (Kishi & Yamaguchi, 1994).

Copper

Severe Cu deficiency is known to cause skeletal abnormalities. Osteoporosis is associated with the genetically-determined malabsorption of Cu that occurs in Menkes' disease (Danks, 1987). The role of Cu in bone metabolism can be linked primarily to the Cu-dependent enzyme lysyl oxidase, for which Cu acts as a cofactor. Lysyl oxidase is required for the formation of lysine-derived cross-links in collagen and elastin (Rucker *et al.* 1998). Studies in laboratory animals have shown that the activity of this enzyme increases in response to an increase in dietary Cu intake (Opsahl *et al.* 1982).

Cu also plays a key role in the inhibition of bone resorption, through its action as a cofactor for superoxide dismutase, which is an antioxidant enzyme containing two atoms of Zn and Cu which acts as a free radical scavenger, neutralising the superoxide radicals produced by osteoclasts during bone resorption. Research in Cu-deficient animals indicates that a lack of Cu results in an inhibition in osteoclast function, but no change in osteoblast activity (Rico, 1991).

Dietary intake of zinc and copper in the elderly

One factor contributing to bone loss in the elderly may be subclinical Zn and/or Cu deficiency. A recent national survey of nutrient intake in older adults in the USA revealed that both the Cu and Zn intakes of elderly men are suboptimal (Ma & Betts, 2000). Data from 3000 men and women aged 60–90 years were reported. For both men and women 75 % failed to meet their respective dietary recommendation for Zn intake (15 mg/d for men and 12 mg/d for women), and none of them reached the lower value of the range recommended for dietary Cu intake (estimated safe and adequate daily dietary intake 1·5–3·0 mg/d). These data support previous reports of poor dietary trace mineral intake in the elderly (Vir & Love, 1979; Sandstead *et al.* 1982; Strain, 1988). To compound this problem the capacity to absorb nutrients appears to decline with ageing (Thomson & Keelan, 1986). Turnlund *et al.* (1986) demonstrated that elderly subjects had reduced fractional Zn absorption and reduced dietary Zn intake compared with young subjects. Ali *et al.* (1998*b*) also reported a significantly reduced fractional Zn absorption in the elderly ($P<0·05$), although this finding has not been consistent (Couzy *et al.* 1993). Using a compartmental model to analyse plasma enrichment following simultaneous oral and intravenous doses of stable isotopes of Zn, Ali *et al.* (1998*a*) reported that the fractional rate of Zn transfer between the gastrointestinal compartment and the plasma was 51 % lower for the elderly group compared with the young control group.

Table 1. Characteristics of patients participating in the study (Medians and ranges for twelve subjects)

	Median	Range
Age (years)	68	62–77
Period since menopause (years)	17	11–36
Ca intake (mg/d)	1013	660–1463
BMI (kg/m^2)	25·1	20·4–29·1

We have recently completed a study of Zn and Cu status in a group of elderly women, diagnosed with osteoporosis according to the World Health Organization (1994) criteria. The subject characteristics are given in Table 1. Measurements were made at baseline and after a 1-month period of supplementation with Ca (Calcichew; Shire Pharmaceuticals, Andover, Hants., UK; 1 g Ca as $CaCo_3$/d). A dietary analysis was carried out during the baseline period using a 4 d weighed diet intake diary, analysed using a computer database (Diet 5; Robert Gordon University, Aberdeen, UK). Three of the twelve women failed to meet the UK reference nutrient intake for Zn of 7 mg/d (Garrow *et al.* 2000) and eleven women failed to meet the US recommended dietary allowance for Zn (Garrow *et al.* 2000). Eight women failed to meet the UK reference nutrient intake for Cu of 1·2 mg/d (Garrow *et al.* 2000) and none met the US estimated safe and adequate daily dietary intake (Garrow *et al.* 2000). One of the patients failed to complete the protocol. Fractional Zn absorption was determined in this group using a dual-stable-isotope technique. Two different stable isotopes of Zn were administered, one orally (^{67}Zn) and one intravenously (^{70}Zn), following a standard breakfast meal. The mean fractional Zn absorption was 0·21 (SD 0·048), which is significantly lower than that reported in a group of healthy young women using the same technique and the same test meal (mean fractional Zn absorption 0·30 (SD 0·1); $P<0·05$; Fig. 1; Lowe *et al.* 2000).

Is zinc and copper status suboptimal in the elderly?

Whether or not suboptimal dietary Zn or Cu intake, coupled with diminished absorption, results in subclinical deficiency in the elderly is difficult to assess, due to the lack of specific and reliable biochemical indicators of Zn and Cu status. Plasma or serum concentrations are commonly used as indicators of status, but are notoriously unreliable because they can be affected by factors unrelated to body levels, such as medication, including hormone-replacement therapy, diuretics and laxatives. This unreliability is reflected in the inconsistencies in the literature regarding

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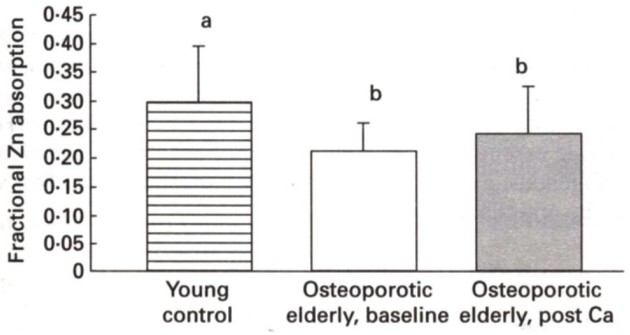

Fig. 1. Fractional zinc absorption in young women (control group; ▤; *n* 6) and osteoporotic elderly women at baseline (□; *n* 11) and after a calcium supplementation period of 1 month (■). Values are means and standard deviations represented by vertical bars. [a,b]Mean values with different superscript letters were significantly different (ANOVA, Tukey *post hoc*; $P<0·05$).

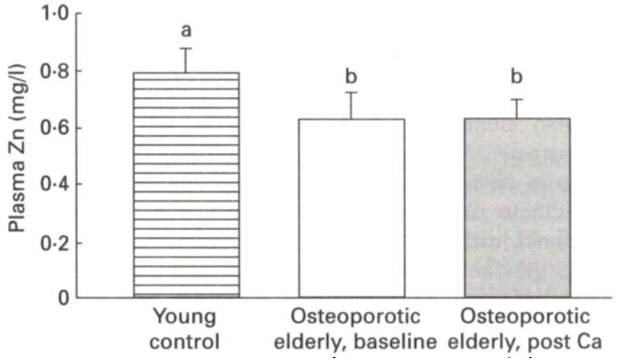

Fig. 3. Plasma zinc concentration in young women (control group; ▤; *n* 13) and osteoporotic elderly women at baseline (□; *n* 11) and after a calcium supplementation period of 1 month (■). Values are means and standard deviations represented by vertical bars. [a,b]Mean values with different superscript letters were significantly different (ANOVA, Tukey *post hoc*; $P<0·05$).

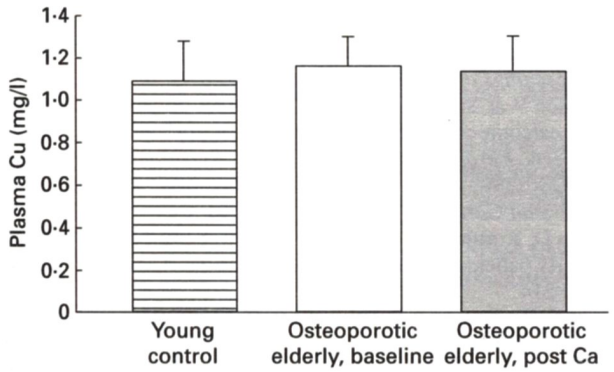

Fig. 2. Plasma copper concentration in young women (control group; ▤; *n* 13) and osteoporotic elderly women at baseline (□; *n* 11) and after a calcium supplementation period of 1 month (■). Values are means and standard deviations represented by vertical bars.

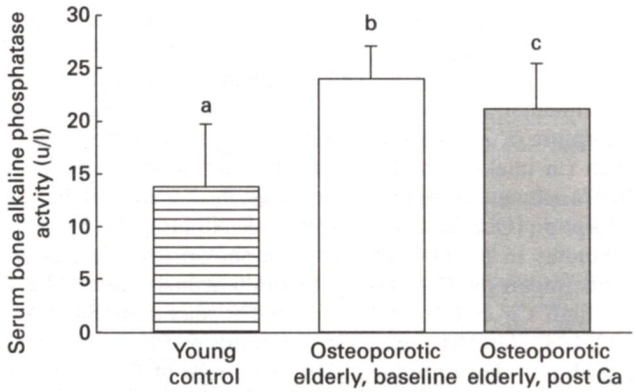

Fig. 4. Bone specific alkaline phosphatase activity in young women (control group; ▤; *n* 13) and osteoporotic elderly women at baseline (□; *n* 11) and after a calcium supplementation period of 1 month (■). Values are means and standard deviations represented by vertical bars. [a,b,c]Mean values with different superscript letters were significantly different (ANOVA, Tukey *post hoc*; $P<0·05$).

plasma levels of these minerals in the elderly; some researchers have reported reduced levels (Wood, 1990), while others have reported the reverse (Relea *et al.* 1995; Wapnir, 1998). Other biochemical indices of Zn status include determination of the activity of Zn-dependent enzymes, such as alkaline phosphatase. In our own study described earlier in which none of the women was taking medication or mineral supplements, there was no significant difference in plasma Cu concentration compared with a control group of young healthy women (*n* 13; Fig. 2). In contrast, plasma Zn concentration was significantly lower ($P<0·05$) and serum alkaline phosphatase activity was significantly higher ($P<0·05$) in the elderly osteoporotic group (Figs. 3 and 4). The high alkaline phosphatase activity illustrates the problems associated with the interpretation of biochemical data in elderly patients. Although serum alkaline phosphatase activity has been shown to respond to changes in dietary Zn intake in young subjects (Weismann & Høyer, 1985), in osteoporosis, where bone turnover is elevated (both resorption and mineralisation), alkaline phosphatase activity is raised independently of Zn status.

Rather than draw conclusions from indices of status, perhaps the question we should be asking is: do dietary supplements of Zn and Cu reduce bone loss in osteoporotic patients? The most compelling evidence to suggest that they do comes from the work of Saltman & Strause (1993). They conducted a double-blind placebo-controlled study in which a group of 137 post-menopausal women were divided into four groups. Each group received a placebo, Ca (250 mg, four times daily), trace minerals (mg/d Zn 15, Cu 5, Mn 2·5) or both Ca and trace minerals. Bone mineral density was measured at baseline and after 2 years of the supplementation regimen. The results revealed that the percentage changes in bone mineral density were: placebo −2·23, trace minerals −1·66, Ca −0·5, Ca + trace minerals +1·28. The change in bone mineral density in the group treated with Ca and trace minerals was significantly different from that for the placebo group ($P=0·036$), clearly supporting a therapeutic role for Zn and Cu in the treatment of osteoporosis. Further support comes from the work of Delmi *et al.* (1990),

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who reported that the clinical outcome of elderly patients with femoral neck fracture is improved by dietary supplementation with a mixture of minerals, vitamins, protein, carbohydrate and lipids. Research in experimental animals has also demonstrated a therapeutic role for Zn in osteoporosis. Yamaguchi (1995) performed a series of studies *in vivo* and *in vitro* on a Zn compound, β-alanyl-L-histidinato zinc. This compound, in which Zn is chelated to β-alanyl histidine, was found to completely prevent bone loss in the femur of ovariectomised rats. Analysis of the mechanism in cell culture revealed that β-alanyl-L-histidinato zinc stimulated proliferation and differentiation of osteoblastic cells, inhibited bone resorption and stimulated calcification.

Influence of calcium on zinc and copper absorption

In the context of the present review on the putative benefits of trace mineral supplementation, it is important to discuss potential interactions between nutrient supplements. Ca supplements, with or without vitamin D, are often prescribed for the treatment of osteoporosis. Paradoxically, Ca supplementation may accentuate the problem of reduced Zn and Cu levels. It is well established from research in experimental animals that phytic acid decreases the absorption of Zn. This inhibitory effect is more pronounced when Ca intake is high, due to the formation of insoluble Zn–Ca–phytate complexes, rendering the Zn unavailable for absorption (Oberleas, 1966; Davies & Nightingale, 1975).

Studies in human subjects have shown that Ca supplements impair the absorption of simultaneously-ingested Zn, and high Ca and P intakes lower Cu retention (Snedeker *et al.* 1982; Grekas *et al.* 1988; Argiratos & Samman, 1993). In addition, dairy products rich in sources of Ca have also been shown to significantly reduce Zn absorption ($P < 0{\cdot}05$; Pecoud *et al.* 1975). In our own study of elderly osteoporotic women we failed to demonstrate any significant effect of Ca supplementation (1 g/d) on the fractional absorption of stable isotopes of Zn, when taken together with a standard meal (Fig. 1). In addition, a 1-month period of Ca supplementation had no significant effect on plasma Zn or Cu concentrations (Figs. 2 and 3).

Concluding remarks

It is well established that Cu and Zn are essential for bone mineralisation and osteoblast function. It appears that some elderly groups may have reduced dietary intakes and/or absorption of these minerals, and that dietary supplements of Zn and Cu can effectively reduce the rate of bone loss. There are still some unanswered questions, particularly regarding trace mineral status in the elderly and in those with osteoporosis. The use of stable isotopes, coupled with compartmental modelling, has been shown to provide a novel approach to the study of changes in trace mineral metabolism and whole-body status. Models of Zn metabolism have enabled the effect of acute dietary Zn depletion on homeostasis to be investigated in young subjects (King *et al.* 2001). Modelling of stable Cu isotope data has been useful in investigating the changes in Cu metabolism that occur in patients with Wilson's disease, as a tool to aid diagnosis (Lyon *et al.* 1995). Application of this approach to investigate changes in mineral metabolism that occur due to ageing and in patients with osteoporosis will enable the identification of sites of altered mineral metabolism, and provide essential information on status, absorption and also endogenous excretion, so that supplementation regimens can be optimised.

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